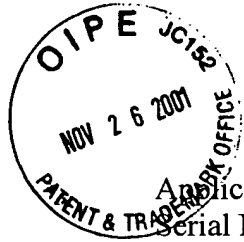


S1237/7011
Declaration of K. Walsh
Page 1

Unsigned



ATTORNEY'S DOCKET NO: **S1237/7011**
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Kenneth Walsh
Serial No: 09/408,905
Filed: September 29, 1999
For: AKT Compositions for Enhancing Survival of Cells
Examiner: Nickol, G.
Art Unit: 1642

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20
LD
12-7-21

Commissioner for Patents
Washington, D.C. 20231

Declaration of Kenneth Walsh Under 37 CFR §1.131

Sir:

I, Kenneth Walsh, declare that:

1. I am an inventor of the above-identified patent application. I make this Declaration in support of that application and in response to the Office Action (Paper No. 16) dated May 14, 2001.
2. The purpose of this Declaration is to establish conception and diligence in reduction to practice of the claimed invention in the United States as of a date prior to October 17, ¹⁹⁸⁷~~2001~~, which is the publication date appearing on the S. Datta et al. reference, entitled "Akt Phosphorylation of BAD Couples Survival Signals to the Cell-Intrinsic Death Machinery" (Cell, vol. 91:231-241 (October 17, 1987), made of record by the Examiner during prosecution of this application.
3. The claimed invention is directed to a method for treating myocardial infarction by administering an Akt molecule.

4. The Summary of the Invention (application page 2, lines 16-22) states:

“The invention involves the discovery that Akt (also known a Protein Kinase-B, PKB) inhibits apoptotic cell-death of cells, and in particular, inhibits apoptotic cell-death of cardiomyocytes, skeletal myocytes and/or vascular endothelial cells. In view of these discoveries, it is believed that Akt molecules can be used to inhibit apoptotic cell-death of the afore-mentioned cell types, and in particular, to treat conditions (e.g., myocardial infarction) that result in increased apoptotic cell-death of cardiomyocytes, skeletal myocytes and/or vascular endothelial cells.”

5. To establish conception of the claimed invention, I submit herewith a reproduction of two pages from the laboratory notebook of Yasushi Fujio (Exhibit A, attached hereto) who worked in my laboratory under my direction on research relating to the claimed invention. The two notebook pages are entitled “C2C12 cells 1” and “C2C12 cells 2,” and are dated “971116” and “971119,” respectively (top right hand corner of pages, year/month/day). These experiments describe the results of experiments which establish that Akt inhibits apoptotic cell-death of myocytes.

6. The experimental strategy for the Exhibit A experiment is presented in Example 1 of the application as filed (pages 32-40, “Akt controls skeletal myocyte viability”). Example 1 of the application further describes the materials and methods that are identified in Exhibit A (e.g., “C2C12 cells” (page 33, line 10); “Lipofectamine” (page 34, line 30); “OptiMEM” (page 34, line 30); “CMV” (page 35, line 11, “cytomegalovirus promoter”); “β-Gal” (page 35, line 17, “β-galactosidase”); “Akt(wt)” (page 35, line 22, “wild-type Akt”); and “Akt(K179M)” (page 35, line 23).

7. The results shown in Exhibit A (page 2 table and bar graph) also are described in Example 1 of the application as filed, "... these data show that Akt is effective in protecting mitotic cells against death during the differentiation process" (page 40, lines 1-2).
8. The above-identified patent application is substantially identical to the provisional application (USSN 60/102,740, filed October 2, 1998) to which it claims priority. To show diligence between conception and the filing date of the priority document, I also submit a reproduction of four additional pages from the laboratory notebook of Yasushi Fujio (Exhibit B, attached hereto) who worked in my laboratory under my direction on research relating to the claimed invention. The four notebook pages are entitled "Endothelial cell 1", "Endothelial cell 2", "cardiomyocyte 1", and "cardiomyocyte 2", with the earliest dates for these experiments being "960407" for the endothelial cell experiments and "980630" for the cardiomyocytes, respectively (top right hand corner of pages, year/month/day). These experiments describe the results of experiments which establish that Akt inhibits apoptotic cell-death of endothelial cells and cardiomyocytes, respectively.
9. Exhibit A establishes a date of conception of the invention prior to October 17, 1997, which is the publication date appearing on the S. Datta et al. reference.
10. Exhibit B and the above-identified application and priority document as filed establish due diligence in reducing the invention to practice from a date prior to the publication date appearing on the S. Datta et al. reference to the filing date of the above-identified patent application priority document.

11. From these exhibits, one of ordinary skill in the art would recognize that I had conception of the invention in this country from a date prior to October 17, 1997, and that reduction to practice was diligently pursued from conception until the filing of the patent application priority document.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: _____

Kenneth Walsh
207 Judy Farm Road
Carlisle, MA 01742

Exhibit A
(2 pages)

USSN 09/408,905
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C2C12 cells 1

971116

C2C12 cell

sol (A)	①	②	③
	CMD-basic 8 P	CMD-Akt 8 P	CMD-Akt (K179M) 8 P
	β -Gal 2	2	2
	OptiMEM 240	240	240

sol (B)	Lipofectamine	90 μ l
	OptiMEM	810 μ l

sol (A) 250 μ l } incubate at r.t. for 45 min
 sol (B) 250

↓
 add 2ml of GH (without antibiotics)

↓
 500 μ l / well

↓
 O/N

↓
 DM for 2 days

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C2C12 cells 2

971119

cont vector

Akt (wt)

Akt (K179M)

42

89

59

49

91

54

39

70

42

50

93

57



x10

x10

45

88.7

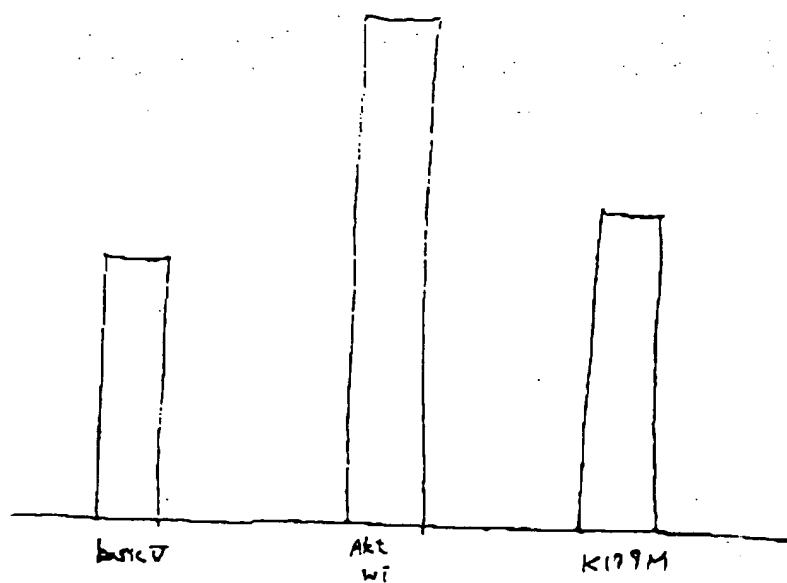
54.5

25.4

210.6



pup again



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Exhibit B
(4 pages)

USSN 09/408,905

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CRISTALLINE CELL 2

980406

Ad. HA Adt : 10^{10} pfu/mlAd. β -gal : 10^9 pfu/ml

MOI : 30

HUDEC

 2×10^4 cells / 500 μ l 5×10^5 cells / 12.5 ml 1.5×10^7 pfuAd. HA Adt : 1.5 μ lAd. β -gal : 15 μ l↓
DN

↓

cultured for 24 hr

with or without DFCF

↓

cell count

T/E 100 μ lM+Trypan 100 μ l

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Endothelial Cell 2

TEGF stimulation

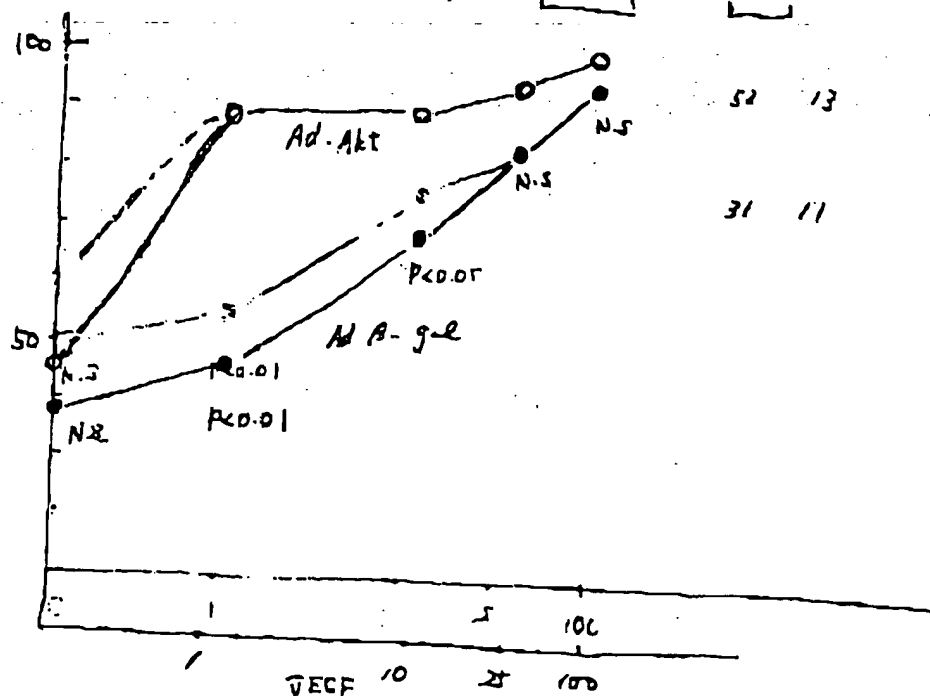
960407

5

960408

20% FCS		x100		25		10		1		0	
P	A	P	A	P	A	P	A	P	A	P	A
51	50	46	45	42	42	27	45	21	38	13	30
58	61	50	54	45	57	32	42	28	41	21	23
69	67	48	49	36	43	36	39	27	50	23	29
58	55	42	49	40	49	42	47	20	44	22	20
59±3.7	58±2.7	46.5±0.7	47±2.8	41±2	47±3	34±3	44±2	23±2	43±3	20±2	26±2
				0.04		0.004					

100±6	96±6	79±3	83±3	69±3	79±5	59±5	74±3	77±3	73±5	33±3	44±3
95±4	100±4	83±4	100±4	89±6	89±4	47±4	87±6	39±4	49±4		



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Contingency 1

980630

cell culture

96 wells

1.5×10^4

0000
0000
0000
0000

p-32
2.5p
0000
0000
0000
0000

mg Akt
5p
0000
0000
0000
0000

Akt
2.5
0000
0000
0000
0000

25~
MOI 50

for no infection

2
0.5p

0000
0000
0000
0000

3p
0000
0000
0000
0000

1.0
0000
0000
0000
0000

10~
MOI 20

infection %

→ serum for 2 days

2×10^6 cells

6 wells

0000
0000

2.5
 $2.5 \times 10^5 \times 6$

0000
0000

$2.5 \times 10^5 \times 6$

3×10^6

0000

0000

Ca⁺⁺ (P)

Epifluorescence

24 well

1×10^5 / well

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Cardiomyocyte 2

980703

K)

Total

0	2.28	12.5	50
16	20	24	24
15	18	20	28
17	17	18	24
14	18	18	25

B

16	16	20	28
17	20	20	29
18	20	18	25
15	15	21	26

Alt

22/22	18	26	24
12	17	24	25
14	18	22	23
14	16	25	21

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